REMARKS/ARGUMENTS

By this Amendment, claims 1, 9, 15 and 17 are amended and claims and 13-14 and 20 are cancelled. Claims 1-12 and 15-19 are pending.

Favorable reconsideration is respectfully requested in view of the foregoing amendments and the following remarks.

Claim Amendments

The claims have been amended to specify that the vaccine is a meningococcal conjugate vaccine prepared using a meningococcal polysaccharide and a meningococcal protein. These limitations have been incorporated from claims 13-14 and 20, which have accordingly been cancelled. No new matter has been added.

Claim Rejection under 35 U.S.C. § 112

The rejection of claims 9 and 17 as allegedly being indefinite in their recitation of the term "substantially" is obviated by the foregoing amendments deleting "substantially" from the claims. Accordingly, reconsideration and withdrawal of the indefiniteness rejection are respectfully requested.

Claim Rejections under 35 U.S.C. §103

Claims 1-4 and 8-14 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over US 6,800,728 (Schwartz), US 5,965,714 (Ryall), and US 4,963,232 (Kuriyama), as evidenced by Behr et al. (Tetrahedron 59, pages 543-553). Claims 1-20 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over US 6,800,728 (Schwartz), US 5,965,714 (Ryall), and US 4,963,232 (Kuriyama), US 5,480,643 (Donovan) and US 5,066,408 (Powell) as evidenced by Behr et al. (Tetrahedron 59, pages 543-553). These rejections are respectfully traversed.

Claimed Meningococcal Conjugate Vaccine Production not Taught in Applied Art

Taken alone or in combination, the cited references do not disclose or suggest all the steps to produce, purify and control meningococcal conjugate vaccines in commercial volumes. The applied art does not describe a method for preparing meningococcal conjugate vaccine using reductive amination reaction of hydrazine and aldehyde. It must be emphasized that known methods utilizing protein modification lead to insolubility and precipitation problems in the final product. The claimed invention thus addresses a need for an improved industrial-scale process for providing a conjugate vaccine that can protect people against meningococcal disease.

Schwartz does not Disclose Meningococcal Vaccine

Schwartz does not disclose vaccines against meningococcal disease.

Schwartz discloses reagents and methods for conjugation of biomolecules to other biomolecules, polymers, metals or drugs to overcome existing limitations and improve the characteristics of reagents and existing methods. The reagents are heterobifunctional compounds having the formula:

B-R-Y

wherein B can be an amino radical which reacts directly with amino forming amide or thiol which reacts directly sulfhydryl groups forming stable thioether bonds; R is a divalent group with various combinations and Y is a hydrazino group, or oxiamino or carbonyl (ketone or aldehyde).

Modified solid supports are provided, including synthetic polymers, beads, glass, slides, metals and particles which have been modified by reaction with bifunctional reagents and that possess a hydrazino or oxyamino group. These modified solid supports are useful in the immobilization of biomolecules modified or not to possess a carbonyl group. The immobilized biomolecules may be used in diagnostic and therapeutic applications.

The methodology is also used for obtaining conjugate vaccines through the reaction of the carrier protein, which was modified with bifunctional reagent (hydrazino or oxyamino; in a neutral to alkaline pH (ranging from 7-9); during the period from 1 to 4 hours). The bifunctional reagent is an organic compound (thiochloridrate pirimidinic) with bacterial polysaccharide (PS) that has been oxidized to contain aldehyde groups. The first reagent binds to the protein through amino groups available (lysine residues) and, then, promotes the exposure of the amino group. It is unclear how it would be the utilization of the second reagent in the activation of the PS.

The conjugating reaction occurs by amination without the use of reducing agent (which is required for reduction of double bonds formed).

The reaction of activation of protein through available amine groups (lysine residues) with subsequent exposure of the amino group is generally not applied to bacterial anatoxin used as carrier proteins (Tetanus and Diptheria Anatoxins cited in Schwartz) for the production of conjugate vaccines because these proteins must be subjected to the process of detoxification with formaldehyde, which hinders these groups.

The heterobifunctional organic compounds used are toxic substances and they can cause adverse reactions if used for producing products for human use as vaccines. Moreover, they are spacers between the two biomolecules and they introduce undesirable antigenic radicals into the structure of the conjugated (aromatic groups). In addition they can overload the immune system if used as a vaccine and induce reduced immune response (Schwartz points out this fact at column 20, lines 8-12).

Not using reducing agents such as sodium cyanobohydrite usually employed in conjugation reactions by reductive amination, can generate greater instability in the structure of the conjugate due to the possible molecular rearrangements caused by the presence of double bonds (by resonance effect), which could provide inter-molecular reactions.

Schwartz does not show process times or quality control of the intermediate and final products.

In addition, the scale of batches of the conjugate vaccine is not showed, so it is not possible to characterize the development of the process for the production of the vaccine in industrial scale.

Finally, Schwartz does not show tests to evaluate the immunogenicity and toxicity of its conjugate vaccine in an animal model.

Thus, it must be emphasized that the method of Schwartz reflects the state of the art of conjugating reagents, which have serious technical drawbacks that make unpractical the synthesis at industrial scale of a conjugate vaccine.

Ryall

In Ryall the polysaccharides, regardless of the chemical structure, are depolymerized from a hydrolysis reaction with hydrogen peroxide to generate the oligosaccharides with uniform size. Such oligosaccharides react with hydrazine or amine, with the aim of introducing amine groups on the oligosaccharide.

This reaction, known as a derivatization reaction, can occur in the presence of EDAC, since the reaction of depolymerization of the polysaccharides originates chemical groups capable of reacting with hydrazide and/or amine in the presence of EDAC. The polysaccharide and/or derivatized oligosaccharide can selectively react with carboxylic groups of the protein.

The process of depolymerization of the polysaccharide as described in Ryall is based on an adaptation of the reaction of carbohydrate degradation by hydrogen peroxide in alkaline medium (Isbell, H. S. et al., 1987) in which the depolymerization reaction occurs from a random attack of H_2O_2 at glycosidic bonds, resulting in depolymerized carbohydrate chains with uniform distribution of molecular weight.

According to the mechanism proposed by Ryall for the depolymerization reaction, two reactive groups are formed: aldehyde groups and carboxylic acid groups. Aldehyde groups react directly with hydrazides, while the carboxylic acid groups react only with hydrazides when they are in the presence of EDAC in the derivatization step of the oligosaccharide. The bond between the hydrazide or amine with the oligosaccharide can be stabilized by the action of a reducing agent such as $NaBH_3CN$.

For the conjugation reaction, Ryall teaches the use of a spacer agent between the polysaccharide and the protein. The spacer agent is attached to the polysaccharide and, then, the spacer agent is attached to certain amino acids of the protein, in a selective manner. Thus, the depolymerized and hydrazide-or-amine-derivatized polysaccharide can be directly linked to the carboxylic acid groups of the protein in the presence of EDAC or EDAC + N-hydroxysuccinimide.

There is also the possibility of performing a second derivatization in the polysaccharide (depolymerized and derivatized with hydrazide or amine) using reagents of bifunctional combination (molecules that have two distinct (terminal) reactive moieties, which are capable to link to different molecules such as amine or thiol groups).

The process of depolymerization of the polysaccharide according to Ryall occurs randomly in relatively drastic conditions, using high temperatures (30 to 80 °C) which could lead to degradation of thermolabile molecules such as polysaccharides of Neisseria meningitides of group A. In this reaction (in addition to the aldehyde groups capable of reacting directly with the hydrazide or amine – step of derivatization of oligosaccharides) are generated carboxylic groups (COOH), which require the presence of EDAC in the step of derivatization.

Obtaining modified polysaccharides for the conjugation process with the carrier protein consists of two steps: (1) a depolymerization reaction itself; and, (2) an activation

(derivatization) of the depolymerized polysaccharide. The duration of the two stages can reach up to 52 hours.

After these two steps, the conjugating reaction occurs, for 24 to 27 hours. Thus, the total time for obtaining a batch of conjugate vaccine is 79 hours. The process times of Ryall are quite high, which would dissuade a person of ordinary skill in the art from consulting or combining the teachings of Ryall to develop a method of producing meningococcal vaccine on an industrial scale.

Moreover, the use of spacers between the two biomolecules to be conjugated causes the introduction of undesirable antigenic radicals into the structure of the conjugate. This feature can overload the immune system (if used as a vaccine) and induce an immune response reduced to the saccharidic antigen of interest.

The purification step using saline precipitation is coarse and cumbersome.

The scale of all batches of conjugate vaccine is not shown, so it is not possible to characterize the development of the whole process to obtain vaccines on an industrial scale.

Tests for evaluating the toxicity and immunogenicity of conjugate vaccines animal model are not presented.

Additional References do not Remedy Aforementioned Deficiencies

Kuriyama describes the purification of hydrazine in order to reduce the total organic carbon (TOC) concentration. The process of Kuriyama comprises two distilling steps.

Kuriyama describes the synthesis of the reagent and does not describe its use in processes of production of conjugate vaccines.

Regardless of whether or not the Office is correct that Donovan and Powell teach the use of sodium carbonate as a buffer, these references still fail to remedy the aforementioned deficiencies of the other references to teach all the limitations of the claimed invention.

Accordingly, reconsideration and withdrawal of the obviousness rejections are respectfully requested.

For at least the reasons set forth above, it is respectfully submitted that the above-identified application is in condition for allowance. Favorable reconsideration and prompt allowance of the claims are respectfully requested.

Should the Examiner believe that anything further is desirable in order to place the application in even better condition for allowance, the Examiner is invited to contact Applicants' undersigned attorney at the telephone number listed below.

Respectfully submitted,

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